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The Herzenberg Lecture: How to make a B1 cell?

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Short title: *How to make a B1 cell?*

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This essay presents a short historical perspective on B1 cells and a synopsis of contemporary work in my laboratory on generating B1 cells from B2 cells via B cell receptor exchange.

This lecture is a tribute to a great scientist and longtime friend, Len Herzenberg from Stanford University. As a young group leader at the Institute for Genetics in Cologne, founded by Max Delbrück after the war, I had met Len in the early 1970s at a meeting in the German Democratic Republic, and thus began a friendship lasting until Len's death in 2013. Naturally, this included Lee Herzenberg, the central person at this first World Congress on B1 cells here in Tarrytown, New York. For a long time, Len's and Lee's house in Palo Alto became a second home for me, and I can remember many endless discussions with Lee about her beloved B1 cells deep into the night. Len was not only a famous geneticist and immunologist, but also a wonderful human being of a limitless curiosity and broad worldview, and one of the first scientists from the U.S. in my field actively supporting and helping the new generation of scientists in postwar Germany. It was through him that I realized early on the power of fluorescence-activated cell sorting in combination with monoclonal antibodies, and it was with his help that we acquired, at our young institute in Cologne, what I think was the fourth FACS machine produced by Becton-Dickinson. In technical terms, this provided the basis for our own work on B1 cells, which was of course inspired by the work of the Herzenbergs and their colleagues.

From the very beginning, the relationship of B1 and the classical B2 cells was at the center of our interest in that context, as it was for most others in the field. Did B1

cells represent a separate B cell "lineage," perhaps of fetal origin, or was B1 differentiation driven by the engagement of certain self-antigens with a particular set of B cell receptor (BCR) specificities recognizing epitopes shared by common pathogens in the environment, and thus recruited into an innate system of first-line defense? While not mutually exclusive, the latter view was supported by evidence for the selection of particular BCR specificities in the B1 cell compartment, and the dominant contribution of these cells to natural antibodies in the blood (for review of the older literature, see Refs. 1–3). While the field has moved forward substantially over the past decades, as can be seen by comparing the proceedings of the 1992 conference on B1 cells published at the time in *Annals of the New York Academy of Sciences*[4] with the proceedings of the 2014 conference also published in *Annals*[5], several of the issues mentioned above have still not been fully resolved, one of them being the role of BCR specificity and self-antigen recognition in the acquisition of the B1 cell phenotype. This problem has occupied myself and collaborators in my group over many years, and most of my lecture at the Merinoff World Congress 2014: B-1 Cell Development and Function in Tarrytown, New York[a]— summarized below—dealt with this work, which is still ongoing.

Pathways of B1 cell generation

Early work of my group had shown that B1 cells do not only arise from fetal but also from postfetal progenitors. This became apparent from the accumulation of N sequences at the V–D and D–J borders of VDJ rearrangements carried by B1 cells at different times in ontogeny. Whereas such sequences are essentially absent from VDJ rearrangements of B cells isolated from fetal or newborn mice, they accumulate in both B1 and B2 cells subsequently, albeit at lower tempo in the B1 compartment.[6,7] This is due to the postnatal expression of TdT in the cells, a developmental feature shared between B1 and B2 cells. While this result did not directly address the B1/B2 lineage problem, it would be compatible with the notion that B1 cells could be generated in two different ways, namely, (1) from a perhaps distinct fetal B1 cell precursor and (2) from B2 cells through a particular type of activation through the BCR.

[a] http://molmed.org/events/world-congress/2014

At that time, conditional gene targeting had been established in my laboratory in Cologne, and we decided to use this approach to genetically address whether a B2 cell could be converted into a B1 cell by exchanging its BCR with a B1-typical BCR that recognized an epitope shared among self-antigens and bacterial pathogens. This work was initiated in Cologne by Kong Peng Lam, continued at Harvard Medical School by Jane Seagal and Kevin Otipoby, and is presently being pushed ahead by Robin Graf, after my move from Boston to the Max Delbrück Center at the end of 2011 (a manuscript describing the results should be submitted for publication within this year).

At this stage, the results of the work in progress can be summarized as follows: upon the induced exchange of a B2-typical transgenic BCR (through which mature B cells had been generated *in vivo*) with a phosphatidyl-choline (PtC)–specific B1-typical BCR (whose expression in the germ line results in a pure B1a B cell compartment in the mouse), the PtC-specific B cells undergo several rounds of rapid cell division *in vivo* over a period of a few days, and then persist as resting cells in the spleen and, abundantly, the peritoneal cavity of the recipient (syngeneic) animals. Significantly, the cells phenotypically convert to a B1a phenotype in terms of cell surface and other markers, and exhibit B1a-typical survival and response properties.

While we do not know whether and to what extent B2-to-B1 cell conversion plays a role in normal physiology, as a reflection of the response of B cells to particular stimuli, the data clearly indicate that B2 cells have the capacity to convert to B1 cells both phenotypically and, at least in part, functionally. It is tempting to think that the mechanisms controlling this differentiation process similarly operate in fetal B1 cell differentiation. Perhaps, as discussed by David Nemazee at the 2014 conference, B1 lineage progenitors (for whose existence there is now strong evidence[5]) have a propensity to respond to certain self-antigens in the sense of positive selection rather than tolerance, like immature B2 cells; while mature B2 cells behave like B1 progenitors if exposed to these same antigens.

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Conflict of interest
The author declares no conflicts of interest.

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